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DESCRIPTION CN108403930A

A compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine, its preparation method, and its application.

[0001]

Technical Field

[0002]

This invention relates to the field of oral preparations, and particularly to a compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine, its preparation method, and its application.

[0003]

[0004]

Studies have shown that changes in the gut microbiota can lead to obesity. The mechanism is as follows: after high-calorie foods are chewed by the teeth and initially broken down by the stomach, they enter the intestines, where microorganisms are responsible for completing the remaining digestive work. During the fermentation process, a large amount of acetate is produced. This acetate is absorbed by the intestines, travels through the blood-brain barrier, and enters the brain. The acetate that enters the brain activates the parasympathetic nervous system.

The parasympathetic nervous system, activated by acetate, sends a signal to the pancreas to secrete insulin. As a result, β cells begin to secrete large amounts of insulin, and the cells begin the process of storing energy. At the same time, the parasympathetic nervous system sends a signal to the stomach to release ghrelin and gastrin. As ghrelin is produced in large quantities, hunger follows.

[0005]

In addition, long-term consumption of high-fat foods can lead to gut microbiota imbalance, resulting in higher levels of acetic acid in the gut. This positive regulatory mechanism leads to increased food intake, thereby promoting obesity and insulin resistance.

Changes in the dominant gut microbiota during the development of obesity can further disrupt the gut microbiome.

[0006]

1. The proportion of SCFA-producing bacteria in the intestine (e.g., **Butyrivibrio**, **Bifidobacterium bifidum**, **Bifidobacterium pseudocatenulatum**, **Megasphaera**) was significantly reduced;

[0007]

2. The ratio of Firmicutes to Bacteroidetes in the gut was significantly increased;

[0008]

3. The proportion of beneficial bacteria (such as *Akkermansia muciniphila*, Akk bacteria; *Bifidobacterium*) is significantly reduced;

[0009]

4. A significant increase in the proportion of opportunistic pathogens (such as *Enterobacter cloacae*) can induce an inflammatory response;

[0010]

5. Gut microbiota can inhibit the expression of fasting-induced adipocyte cytokine (Fiaf), increase the activity of adipocyte lipoprotein lipase (LPL), and induce fat deposition.

[0011]

In summary, the gut microbiota, as an important part of the human body's symbiotic relationship, influences the host's nutrition, energy metabolism, and immune status.

The currently validated conclusions include: gut microbiota dysbiosis is involved in the

pathogenesis of metabolic diseases such as obesity and type 2 diabetes, and the mechanisms involved include excessive energy storage and chronic low-grade inflammation caused by metabolic endotoxemia.

[0012]

Therefore, there is a need for an oral formulation that can effectively regulate the gut microbiota and has a high safety profile.

[0013]

Summary of the Invention

[0014]

The purpose of this invention is to provide a compound preparation that increases the ratio of AKK bacteria to pathogenic bacteria in the intestine, which has good safety and can effectively regulate the intestinal flora.

[0015]

Another objective of this invention is to provide a method for preparing a compound preparation that increases the ratio of AKK bacteria to pathogenic bacteria in the intestine. This process is simple and easy to industrialize.

[0016]

Another objective of this invention is to provide an application of a compound preparation that increases the ratio of AKK bacteria to pathogenic bacteria in the intestine. This compound preparation is used to prepare an oral formulation that increases the abundance of AKK bacteria in the intestine and reduces the abundance of pathogenic bacteria.

[0017]

The technical problem solved by this invention is achieved by the following technical solution.

[0018]

This invention proposes a compound preparation to increase the ratio of AKK bacteria to pathogenic bacteria in the intestine, which is prepared from the following raw materials, by mass percentage:

[0020]

Furthermore, in a preferred embodiment of the present invention, the oligosaccharide is selected from at least one of the following combinations: inulin, fructooligosaccharide, xylooligosaccharide, galactooligosaccharide, isomaltooligosaccharide, polydextrose, resistant dextrin, L-arabinose, and konjac glucomannan.

[0021]

Furthermore, in a preferred embodiment of the present invention, the plant extract is selected from at least one of the following combinations: seaweed extract, kelp extract, polygonatum extract, poria cocos extract, wolfberry extract, kudzu root flavonoids and ginseng extract, and psyllium husk.

[0022]

Furthermore, in a preferred embodiment of the present invention, the fungal extract is selected from at least one of a combination of shiitake mushroom extract, cordyceps extract, ganoderma lucidum extract, hericium erinaceus extract, and yeast extract.

[0023]

Furthermore, in a preferred embodiment of the present invention, the raw materials also include Tremella fuciformis extract, and the amount of Tremella fuciformis extract used is 1%-5% of the mass of the fungal extract.

[0024]

Furthermore, in a preferred embodiment of the present invention, the raw materials also include *Dendrobium officinale* extract, which is 1%-5% of the mass of the plant extract.

[0025]

This invention proposes a method for preparing the above-mentioned compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine, which includes the following steps:

[0026]

The product is obtained by mixing and grinding oligosaccharides, plant extracts, fungal extracts, sodium taurate and jujube extract.

[0027]

Furthermore, in a preferred embodiment of the present invention, the method for preparing the plant extract is as follows:

[0028]

The selected plant materials are washed, crushed and mixed, and then extracted with 4-6 times their weight of hot water at 60°C-80°C for 3-4 hours. The extract is then filtered to obtain the extract. The extract is concentrated and then precipitated with ethanol to obtain crude polysaccharide. The crude polysaccharide is separated by chromatography and eluted with

distilled water to obtain polysaccharide solution. The polysaccharide solution is concentrated and then precipitated with ethanol and dried to obtain powdered plant extract.

[0029]

Furthermore, in a preferred embodiment of the present invention, the method for preparing the fungal extract is as follows:

[0030]

After drying the fungal raw material, grind it thoroughly, add 10-20 times the volume of pure water, and extract at 70-80°C for 3-4 hours. Filter to obtain the extract. Centrifuge the extract at 3000-5000r/min for 10-15 minutes and collect the supernatant. Concentrate the supernatant and precipitate it with ethanol. Collect the precipitate, dry it, and obtain the powdered fungal extract.

[0031]

This invention proposes an application of the above-mentioned compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine. The compound preparation is used to prepare an oral formulation that increases the abundance of AKK bacteria in the intestine and reduces the abundance of pathogenic bacteria.

[0032]

The beneficial effects of the compound preparation, preparation method, and application of the present invention for improving the ratio of AKK bacteria to pathogenic bacteria in the intestine are as follows: The compound preparation for improving the ratio of AKK bacteria to pathogenic bacteria in the intestine is prepared from the following raw materials by mass percentage: 21%-64% oligosaccharides; 21%-44% plant extracts; 9%-31% fungal extracts;

0.5%-5% sodium taurate; and 0.5%-5% jujube extract. This compound preparation has good safety and can effectively regulate the intestinal flora.

The compound preparation method involves mixing and grinding oligosaccharides, plant extracts, fungal extracts, sodium taurate, and jujube extract. This process is simple and easy to industrialize.

The application of the compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine according to the embodiments of the present invention is that the compound preparation is used to prepare an oral preparation that increases the abundance of AKK bacteria and reduces the abundance of pathogenic bacteria in the intestine.

[0033]

Detailed Implementation

[0034]

To make the objectives, technical solutions, and advantages of the embodiments of the present invention clearer, the technical solutions in the embodiments of the present invention will be clearly and completely described below.

Unless otherwise specified in the examples, the conditions shall be performed according to the standard conditions or the conditions recommended by the manufacturer.

If the manufacturers of the reagents or instruments used are not specified, they are all conventional products that can be purchased commercially.

[0035]

The following describes in detail the compound preparation, preparation method, and

application of the present invention for improving the ratio of AKK bacteria to pathogenic bacteria in the intestine.

[0036]

This invention provides a compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine. It is prepared from the following raw materials, by mass percentage: 21%-64% oligosaccharides; 21%-44% plant extracts; 9%-31% fungal extracts; 0.5%-5% sodium taurate; and 0.5%-5% jujube extract.

Preferably, the raw materials include: 34%-51% oligosaccharides; 29%-36% plant extracts; 14%-26% fungal extracts; 2%-5% sodium taurate; and 1%-4% jujube extract.

[0037]

The oligosaccharide is selected from at least one of the following combinations: inulin, fructooligosaccharide, xylooligosaccharide, galactooligosaccharide, isomaltooligosaccharide, polydextrose, resistant dextrin, L-arabinose, and konjac glucomannan.

For example, by mass percentage, the raw materials include 1%-10% inulin, 1.5%-10% xylooligosaccharides, 2%-5% galactooligosaccharides, 2%-5% isomaltooligosaccharides, 5%-10% polydextrose, 5%-10% resistant dextrin, 5%-10% L-arabinose and 0.5%-4% konjac glucomannan.

[0038]

Among them, plant extracts are obtained by extracting from plants, and are mostly plant polysaccharides.

In this embodiment, the plant extract is selected from at least one of the following combinations: seaweed extract, kelp extract, polygonatum extract, poria cocos extract,

wolfberry extract, kudzu root flavonoids (i.e., kudzu root extract), ginseng extract, and psyllium husk.

Plantago ovata seed shell is the outer shell of the seeds of Plantago ovata (Latin name: Plantago ovata), a plant belonging to the Plantaginaceae family and the Plantago genus, which can be directly ground into powder for use.

For example, by mass percentage, the raw materials include 5%-10% seaweed extract, 3%-5% kelp extract, 1.5%-5% polygonatum extract, 3%-5% poria cocos extract, 5%-10% wolfberry extract, 1%-3% kudzu flavonoids, and 2.5%-6% psyllium husk.

[0039]

Among them, fungal extracts are obtained by extracting fungi and are mostly fungal polysaccharides.

In this embodiment, the fungal extract is selected from at least one of the following

combinations: shiitake mushroom extract, cordyceps extract, ganoderma lucidum extract, hericium erinaceus extract, and yeast extract.

For example, by mass percentage, the raw materials include 4-10% shiitake mushroom extract, 0.5%-5% cordyceps extract, 0.5%-6% hericium erinaceus extract and 4%-10% yeast extract.

[0040]

In other embodiments, the raw materials may also include tremella extract, wherein the amount of tremella extract used is 1%-5% of the mass of the fungal extract.

[0041]

In other embodiments, the raw materials also include *Dendrobium officinale* extract, which is 1%-5% of the mass of the plant extract.

[0042]

In other embodiments, the raw materials also include flower extracts, wherein the amount of flower extracts used is 2%-10% of the mass of the plant extracts.

The flower extract is selected from at least one of the following combinations: peony extract, honeysuckle extract, and dandelion extract.

[0043]

The compound formulation in this embodiment is made of oligosaccharides, plant extracts,

fungal extracts, sodium taurate and jujube extract. The oligosaccharides, plant extracts, fungal extracts and sodium taurate work synergistically to effectively regulate the ratio of AKK bacteria and pathogenic bacteria in the intestine.

[0044]

This invention provides a method for preparing the above-mentioned compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine, which includes the following steps:

[0045]

The product is obtained by mixing and grinding oligosaccharides, plant extracts, fungal extracts, sodium taurate and jujube extract.

[0046]

Among them, plant extracts can be commercially available high-purity extracts, or they can be prepared according to the following preparation methods:

[0047]

The selected plant materials are washed, crushed and mixed, and then extracted with 4-6 times their weight of hot water at 60°C-80°C for 3-4 hours. The extract is then filtered to obtain the extract. The extract is concentrated and then precipitated with ethanol to obtain crude polysaccharide. The crude polysaccharide is separated by chromatography and eluted with distilled water to obtain polysaccharide solution. The polysaccharide solution is concentrated and then precipitated with ethanol and dried to obtain powdered plant extract.

[0048]

Among them, the fungal extract can be a commercially available high-purity extract, or it can be prepared according to the following preparation method:

[0049]

After drying the fungal raw material, grind it thoroughly, add 10-20 times the volume of pure water, and extract at 70-80°C for 3-4 hours. Filter to obtain the extract. Centrifuge the extract at 3000-5000r/min for 10-15 minutes and collect the supernatant. Concentrate the supernatant and precipitate it with ethanol. Collect the precipitate, dry it, and obtain the powdered fungal extract.

[0050]

Flower extracts can be purchased or prepared using the following methods:

[0051]

Fresh flower raw materials are added to yeast for fermentation, filtered, post-ripened, and clarified to obtain flower and fruit fermentation liquid; the fermentation liquid is filtered and concentrated to obtain powdered fresh flower extract.

[0052]

The extract of *Elaeagnus angustifolia* can be purchased or prepared by the following method:

Weigh *Elaeagnus angustifolia* and place it in a microwave extraction device. Use a microwave power of 500W-900W and a temperature of 70°C. Extract three times. For the first extraction, add 50%-90% ethanol and extract for 10-30 minutes. For the second extraction, add 50%-90% ethanol and extract for 10-30 minutes. For the third extraction, add distilled water and extract for 20-40 minutes.

[0053]

The first and second ethanol extracts were then concentrated to remove the ethanol. The third water extract was then added, mixed, and concentrated to obtain powdered jujube extract.

[0054]

This invention provides an application of the above-mentioned compound preparation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine.

Targeting key factors of intestinal flora imbalance, the compound preparation has the function of significantly increasing the abundance of AKK bacteria in the intestine and reducing the abundance of pathogenic bacteria, thereby normalizing the imbalanced intestinal flora.

Correspondingly, compound preparations can be used to prepare oral formulations that increase the abundance of AKK bacteria in the intestine and reduce the abundance of pathogenic bacteria.

More specifically, compound formulations can be used to prepare oral preparations that improve obesity symptoms in obese individuals.

[0055]

One of the key factors in gut microbiota imbalance is the abundance of beneficial bacteria, and one of the functions of the compound preparation is to significantly increase the abundance of *Akkermansia muciniphila* (AKK bacteria) in the gut, thereby improving obesity symptoms in obese individuals.

[0056]

1. *Akkermansia muciniphila* is a bacterium in the human gut that can degrade mucin. It is negatively correlated with obesity, diabetes, inflammation, and metabolic disorders. The feces of healthy individuals contain a large number of *Akkermansia* extracellular vesicles (AmEVs), which reduce LPS-induced intestinal permeability of Caco-2 cells.

[0057]

2. The compound preparation can significantly increase the AmEV content in the intestine, enhance the tight junction function of the intestine, reduce weight gain, and improve glucose tolerance;

[0058]

3. *Akkermansia muciniphila* can regulate the thickness of intestinal mucus and maintain the integrity of the intestinal barrier, thereby improving intestinal metabolic function;

[0059]

4. *Akkermansia muciniphila* grows attached to the intestinal mucosa (and can also reproduce vigorously during fasting), and can protect the intestine from pathogens through competition;

[0060]

5. *Akkermansia muciniphila* releases various SCFAs, including acetic acid, while degrading mucin, promoting Foxp3⁺Treg, IL-10, and TGF- β to reduce pancreatic inflammation.

[0061]

6. *Akkermansia muciniphila* can induce the expression of fasting-induced adipokine (Fiaf), which can reduce fat storage capacity.

[0062]

Another key factor in gut microbiota imbalance is the proportion of pathogenic bacteria in the gut, and one of the functions of compound preparations is to significantly reduce the proportion of various pathogenic bacteria in the gut, such as *Enterobacter cloacae*, thereby improving obesity symptoms in obese individuals.

[0063]

This invention targets key factors in gut microbiota imbalance, normalizing the unbalanced gut microbiota.

[0064]

1. It reduces the abundance of opportunistic Gram-negative bacteria such as *Enterobacter*

cloacae in the gut, thereby reducing the level of circulating LPS, reducing inflammatory factors, and thus improving insulin sensitivity.

[0065]

2. The compound preparation can improve the intestinal mucosal barrier function and immune function, thereby inhibiting the growth of harmful bacteria in the intestine;

[0066]

3. Reducing the proportion of major LPS-producing bacteria (Proteobacteria, Escherichia) in the gut can decrease intestinal permeability (promoting the expression of tight junction proteins ZO-1, occluding, and Cb-1), inhibit the TNF- α signaling pathway, and reduce the release of pro-inflammatory factors IL-1 and IL-6, which helps maintain the integrity of the

epithelial barrier, reverses intestinal inflammation caused by obesity, and restores immune balance.

[0067]

The features and performance of the present invention will be further described in detail below with reference to embodiments.

[0068]

Example 1

[0069]

This embodiment provides a compound formulation, which is prepared by the following method:

[0070]

Prepare the following ingredients: 630g oligosaccharides (100g inulin, 100g fructooligosaccharides, 100g xylooligosaccharides, 90g galactooligosaccharides, 50g isomaltooligosaccharides, 50g polydextrose, 50g resistant dextrin, 50g L-arabinose, and 40g konjac glucomannan); 230g plant extracts (50g kelp extract, 80g polygonatum extract, 50g poria cocos extract, and 50g wolfberry extract); 120g fungal extracts (60g shiitake mushroom extract, 40g cordyceps extract, and 20g yeast extract); 10g sodium taurate; and 10g jujube extract.

[0071]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate and jujube extract are mixed and ground to obtain the final product.

[0072]

Example 2

[0073]

This embodiment provides a compound formulation, which is prepared by the following method:

[0074]

Prepare the following ingredients: 220g oligosaccharides (50g galactooligosaccharides, 50g isomaltooligosaccharides, 50g polydextrose, 50g L-arabinose, and 20g konjac glucomannan); 430g plant extracts (100g kelp extract, 80g polygonatum extract, 100g poria cocos extract, 100g wolfberry extract, 20g kudzu root flavonoids, and 30g ginseng extract); 300g fungal extracts (100g shiitake mushroom extract, 50g cordyceps extract, 100g hericium erinaceus extract, and 50g yeast extract); 25g sodium taurate; and 25g jujube extract.

[0075]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate and jujube extract are mixed and ground to obtain the final product.

[0076]

Example 3

[0077]

This embodiment provides a compound formulation, which is prepared by the following method:

[0078]

Prepare the following ingredients: 500g oligosaccharides (100g inulin, 100g fructooligosaccharides, 100g xylooligosaccharides, 100g galactooligosaccharides, 100g isomaltooligosaccharides); 300g plant extracts (100g seaweed extract, 100g Polygonatum sibiricum extract, 50g Poria cocos extract, 50g Lycium barbarum extract); 150g fungal extracts

(50g shiitake mushroom extract, 50g Cordyceps sinensis extract, 50g Hericium erinaceus extract); 25g sodium taurate; and 25g Elaeagnus angustifolia extract and 10g Dendrobium officinale extract.

[0079]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate, jujube extract, and dendrobium officinale extract are mixed and ground to obtain the final product.

[0080]

Example 4

[0081]

This embodiment provides a compound formulation, which is prepared by the following method:

[0082]

Prepare the following ingredients: 350g oligosaccharides (50g inulin, 50g fructooligosaccharides, 50g xylooligosaccharides, 50g galactooligosaccharides, 50g isomaltooligosaccharides, 50g polydextrose, 50g resistant dextrin); 350g plant extracts (50g seaweed extract, 50g kelp extract, 100g polygonatum extract, 50g poria cocos extract, 50g wolfberry extract, and 50g ginseng extract); 250g fungal extracts (100g shiitake mushroom extract, 50g cordyceps extract, 50g hericium erinaceus extract, and 50g yeast extract); 25g sodium taurate; 25g jujube extract; and 8g tremella extract.

[0083]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate, jujube extract, and tremella extract are mixed and ground to obtain the final product.

[0084]

Example 5

[0085]

This embodiment provides a compound formulation, which is prepared by the following method:

[0086]

Prepare the following ingredients: 420g oligosaccharides (50g inulin, 50g xylooligosaccharides, 50g galactooligosaccharides, 30g isomaltooligosaccharides, 50g polydextrose, 50g resistant dextrin, 100g L-arabinose, and 40g konjac glucomannan); 320g plant extracts (50g seaweed extract, 50g kelp extract, 50g polygonatum extract, 30g poria cocos extract, 60g wolfberry extract, 30g kudzu root flavonoids, and 50g psyllium husk); 200g fungal extracts (40g shiitake mushroom extract, 40g cordyceps extract, 40g hericium erinaceus extract, and 40g yeast extract); 20g sodium taurate; and 40g jujube extract.

[0087]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate and jujube extract are mixed and ground to obtain the final product.

[0088]

Example 6

[0089]

This embodiment provides a compound formulation, which is prepared by the following method:

[0090]

Prepare the following ingredients: 420g oligosaccharides (50g inulin, 50g xylooligosaccharides, 50g galactooligosaccharides, 30g isomaltooligosaccharides, 50g polydextrose, 50g resistant dextrin, 100g L-arabinose, and 40g konjac glucomannan); 320g plant extracts (50g seaweed extract, 50g kelp extract, 50g polygonatum extract, 30g poria cocos extract, 60g wolfberry extract, 30g kudzu root flavonoids, and 50g psyllium husk); 200g fungal extracts (40g shiitake mushroom extract, 40g cordyceps extract, 40g hericium erinaceus extract, and 40g yeast extract); 20g sodium taurate; 40g jujube extract; and 30g peony flower extract.

[0091]

The above-mentioned oligosaccharides, plant extracts, fungal extracts, sodium taurate, jujube extract, and peony flower extract are mixed and ground to obtain the final product.

[0092]

The efficacy of the compound formulations in the embodiments of the present invention will be verified through animal experiments.

[0093]

I. Analysis of gut microbiota at the genus and species levels

[0094]

(1) Laboratory animals:

[0095]

Female BALb/c mice, 4 weeks old (weighing 20 ± 1 g), SPF grade, were purchased from the Hubei Provincial Experimental Animal Research Center.

They are housed in an SPF-grade animal facility.

The mice were fed both a regular diet (10% fat calories) and a high-fat, high-protein diet (over 40% fat calories) purchased from the Hubei Provincial Animal Research Center. The mice had free access to water during the feeding period.

[0096]

(2) Grouping and Intervention:

[0097]

After one week of acclimatization feeding, the mice were randomly divided into three groups of 12 mice each:

[0098]

1. Blank control group: continuously fed with ordinary feed;

[0099]

2. Conditional control group: After being fed a high-fat diet for 6 weeks, the diet was switched to a regular diet for 1 week;

[0100]

3. Experimental group: After being fed a high-fat diet for 6 weeks, the mice were switched to the compound preparation of Example 5. Each mouse was gavaged at a dose of 1g/kg per day for 1 week.

[0101]

Mouse sample collection: After dissection of the mice, fresh fecal matter from the intestines was collected into appropriately labeled cryovials and stored at -80°C for later use.

[0102]

(3) Establishment of an obese mouse model:

[0103]

Table 1 Obese mouse model

[0105]

*P<0.05

[0106]

The results of inducing the mouse obesity model show that the weight changes in the control group and the experimental group after 6 weeks of feeding with a high-fat diet ($P < 0.05$) were significantly greater than those in the control group; compared with the blank group, a weight increase of $\geq 20\%$ can be regarded as a typical mouse obesity model.

[0107]

(4) Results Analysis:

[0108]

Table 2. Genus and species levels of gut microbiota in mice of different groups

[0110]

Compared with the blank group, * $P < 0.05$; compared with the conditional control group, # $P < 0.05$; ## $P < 0.05$.01

[0111]

The results showed that the conditional control group fed with high-fat, high-protein diets had a significant decrease in butyric acid-producing bacteria (*Trichophyton* spp.), a significant decrease in *Bacteroides* spp. but an increase in *Bacteroides monomorpha*, a certain increase in *Escherichia coli* abundance, and a significant decrease in *Akkermansia muciniphila*. In summary, the control group was in a state of intestinal disorder with an increase in conditionally pathogenic bacteria and a decrease in intestinal barrier bacteria.

Compared with the control group, the experimental group, after 1 week of intervention with the compound preparation, showed a significant enrichment of *Akkermansia muciniphila* abundance, a decrease in the number of opportunistic pathogens, and a significant increase in SCFA-producing bacteria, indicating a significant enhancement of intestinal immune function.

[0112]

I. Analysis of *Enterobacter cloacae* in the Intestine

[0113]

(1) Laboratory animals

[0114]

Female BALB/c mice, 4 weeks old (weighing 20 ± 1 g), SPF grade, were purchased from the Hubei Provincial Experimental Animal Research Center.

They are housed in an SPF-grade animal facility.

The mice were fed both a regular diet (10% fat calories) and a high-fat, high-protein diet (over 40% fat calories) purchased from the Hubei Provincial Animal Research Center. The mice had free access to water during the feeding period.

[0115]

(2) Grouping and Intervention

[0116]

After one week of acclimatization feeding, the mice were randomly divided into three groups of six mice each:

[0117]

1. Blank control group: continuously fed with ordinary feed;

[0118]

2. Conditional control group: fed with ordinary feed and treated with *Enterobacter cloacae* bacterial solution by gavage for 1 week, fed with high-fat feed for 4 weeks, and then fed with ordinary feed for 1 week;

[0119]

3. Experimental group: Mice were fed ordinary feed and treated with *Enterobacter cloacae* bacterial solution by gavage for 1 week. After feeding high-fat feed for 4 weeks, they were switched to compound preparation and gavaged at a dose of 1g/kg per mouse per day for 1 week.

[0120]

(3) Experimental procedure:

[0121]

Colonization of *Enterobacter cloacae*: After 1 week of acclimatization feeding, mice in the conditional control group and experimental group were administered *Enterobacter cloacae* strain (*E. cloacae* strain obtained from the microbiology laboratory of Wuhan Jinkairui Biotechnology Co., Ltd.) by gavage. After 7 consecutive days, the colonization of *Enterobacter cloacae* in the intestinal flora was detected.

[0122]

Isolation and identification of *Enterobacter cloacae*: After *Enterobacter cloacae* colonization, fresh fecal samples were collected from mice in the conditional control group and experimental group and routinely inoculated and cultured. Single colony samples were obtained by engraftment agar (ECIA) and biochemical identification was performed.

[0123]

Fecal sample collection: At the end of the mouse experiment, fresh fecal samples were collected from the intestines and stored in appropriately labeled cryovials at -80°C for later use.

[0124]

Extraction of total DNA from gut microbiota: Genomic DNA of fecal bacteria was extracted from all samples according to the instructions of the QIAamp DNASTool Mini Kit and stored at -20°C.

[0125]

16S rRNA analysis of *Enterobacter cloacae*:

[0126]

PCR analysis primers:

[0127]

16SrRNA F: AGAGTTTGATCCTGGCTCAG

[0128]

16SrRNA R:TTACCGCGGCTGCTGGCAC

[0129]

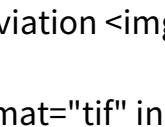
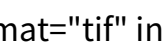
All fecal bacterial genomic DNA samples were subjected to quantitative real-time PCR according to the instructions of THUNDERBIRD qPCR Mix (QPS-201-TOYOBO).

[0130]

454GS FLX sequencing of PCR products: The PCR products were recovered and quantified using the PicoGreen dsDNA Quantitative Detection Kit. According to the sequencing volume

requirements of the samples, they were sent to Wuhan Jinkairui Biotechnology Co., Ltd. for 454GS FLX sequencing.

[0131]

Statistical analysis: All data in this experiment are quantitative data, and all data are expressed as mean and standard deviation . 

[0132]

(4) Results Analysis:

[0133]

① Results of isolation and identification of *Enterobacter cloacae*

[0134]

Samples obtained from fresh fecal samples collected from mice in both the conditional control and experimental groups after colonization with *Enterobacter cloacae* for one week showed that all samples were Gram-negative, short, and crude bacilli under microscopic examination; they were not hemolyzed on blood agar culture; they appeared pink on EMB culture; they appeared pink or red on MacConkey agar culture; and they appeared white and opaque on SS agar culture.

[0135]

Biochemical characteristics of *Enterobacter cloacae*: Oxidase test (+), IMViC test (– – ++), Lysine decarboxylase test (–), Arginine dihydrolase test (+), Ornithine decarboxylase test (+), Sucrose (+), Raffinose (+), Sorbitol (+).

[0136]

Table 3 Biochemical characteristics of *Enterobacter cloacae*

[0138]

As can be seen from the above, the success rate of *Enterobacter cloacae* colonization is 100%.

[0139]

② Abundance analysis of *Enterobacter cloacae* in different groups of mice

[0140]

The results of collecting fecal samples from different groups of mice at different time points were analyzed. The experimental start time was one week after the adaptation feeding: samples were collected in the blank group and the conditional control group in week 6, and samples were collected in the experimental group in week 5 and week 6.

[0141]

Table 4 Abundance of *Enterobacter cloacae*

[0143]

Compared with the blank group, * $P < 0.01$; compared with the conditional control group, # $P < 0.$

01.

[0144]

The results showed that after one course of treatment with the compound preparation, the abundance of the opportunistic pathogen *Enterobacter cloacae* was significantly reduced.

[0145]

The efficacy of the compound formulations of other embodiments of the present invention was verified using the same animal experimental methods described above, and the results showed that the compound formulations of other embodiments of the present invention have the same or similar efficacy.

Based on the genus and species level analysis of the intestinal flora of mice in different groups and the analysis of *Enterobacter cloacae*, it can be seen that the compound bacterial agent of the present invention has the effect of increasing the abundance of beneficial bacteria in the intestine and reducing the abundance of pathogenic bacteria.

Therefore, the compound microbial agent of the present invention can effectively regulate the intestinal flora, thereby improving the obesity symptoms of obese people.

[0146]

The efficacy of the compound formulations in the embodiments of the present invention will be verified through clinical trials.

[0147]

(1) Experimental subjects:

[0148]

Overall characteristics of the study participants: 430 participants were included, of whom 202 were male with a mean age of 34.5 ± 11.2 years and 228 were female with a mean age of 36.9 ± 12.1 years.

All participants in this project were randomly selected from 10 different provinces and regions in China.

[0149]

Existing research indicates that the average body fat percentage (PBF%) for men is 22.7%, lower than women's 28.9%. The standard body fat percentage (PBF%) is as follows:

[0150]

Table 5 Standard Body Fat Percentage

[0152]

Table 6. Criteria for Judging Overweight and Obesity in Adults

[0154]

Table 7. Body type distribution of the participants

[0156]

(2) Intervention and treatment

[0157]

Subjects in different body type groups took the same dose of the compound preparation of Example 6; fasting was required during the period of taking the compound preparation (7 days); before fasting, the diet was adjusted 3 days in advance, mainly consisting of light vegetarian food.

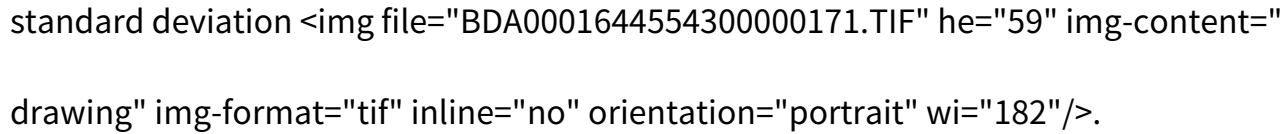
Fasting period: This product completely replaces three meals a day. Take 15g/bag in the morning, noon and evening. The powder should be taken with 37°C warm water. Drink 3-5L of water daily.

After the fasting period, a 7-day refeeding period is followed to resume a normal diet.

[0158]

(3) Data statistics:

[0159]

All data in this experiment are quantitative data, and all data are expressed as mean and standard deviation  drawing" img-format="tif" inline="no" orientation="portrait" wi="182"/>.

[0160]

Table 8. Changes in body fat percentage in different groups

[0162]

*P < 0.05

[0163]

The results showed that the intervention of using the compound preparation to replace the normal diet for 7 consecutive days had a statistically significant effect on fat reduction (P<0.05).

[0164]

In summary, the compound preparation of the present invention, which increases the ratio of AKK bacteria to pathogenic bacteria in the intestine, has good safety and can effectively regulate the intestinal flora; the preparation method of the compound preparation is simple and easy to industrialize.

The application of the compound formulation for increasing the ratio of AKK bacteria to pathogenic bacteria in the intestine according to the embodiments of the present invention is that the compound formulation is used to prepare an oral formulation that increases the abundance of AKK bacteria in the intestine and reduces the proportion of pathogenic bacteria.

[0165]

The embodiments described above are some, but not all, of the embodiments of the present invention.

The detailed description of the embodiments of the present invention is not intended to limit the scope of the claimed invention, but merely to illustrate selected embodiments of the invention.

Based on the embodiments of this invention, all other embodiments obtained by those skilled in the art without inventive effort are within the scope of protection of this invention.